Searching for novel functional amyloids in biofilms

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SEARCHING FOR NOVEL FUNCTIONAL AMYLOIDS IN BIOFILMS

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ABSTRACT

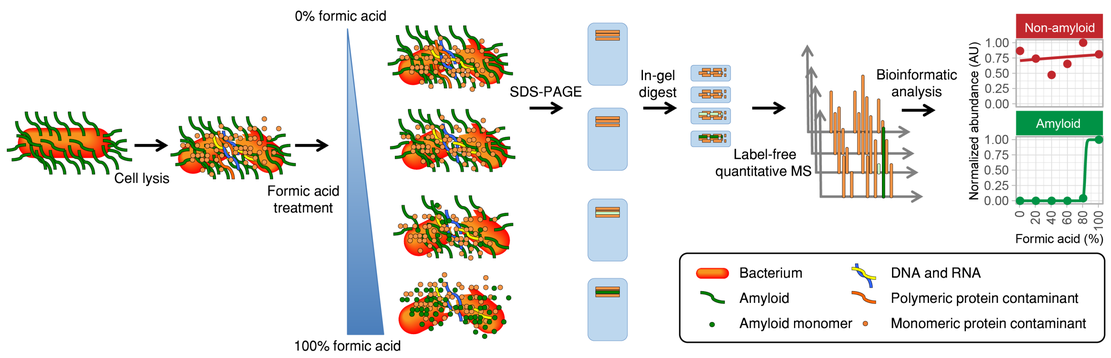
Functional amyloids are important structural and functional components of many biofilms, yet our knowledge of these fascinating polymers is limited to a few examples for which the native amyloids have been isolated in pure form. Isolation of the functional amyloids from other cell components represents a major bottleneck in the search for new functional amyloid systems. However, by taking advantage of the structural stability and the polymeric nature of the amyloids, compared to most other proteins, a new method has been developed to screen for potential amyloids. The method has successfully been evaluated on two established Gram-negative amyloid systems. Proof of concept for the application to complex microbial biofilms was done using activated sludge, where amyloid producing *E. coli* was added and subsequently traced. In addition, another potential novel amyloid candidate was found in the activated sludge biofilm.

AIM

The identification of novel amyloids is key to understand the many roles of amyloids in biofilm structure and function. This study aimed to develop a technique that allows the direct identification of functional amyloids in biofilms and eventually discover novel amyloid candidates.

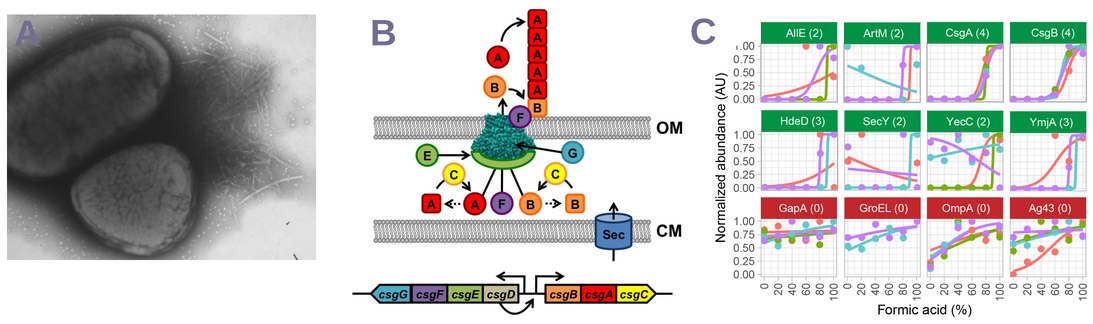
METHODOLOGY

Utilizing formic acid's ability to depolymerize an amyloid fibril, lysed bacteria were freeze-dried and dissolved in increasing concentrations of formic acid (0-100%) and then analyzed for a characteristic increase in protein abundance at a higher percentage of formic acid, where they are dissolved. The proteins were identified using **label-free quantitative LC-MS/MS** using MaxQuant and the MaxLFQ algorithm. Eventually, positive amyloid candidates are identified based on their abundance profiles with respect to the formic acid concentration using an automated script.



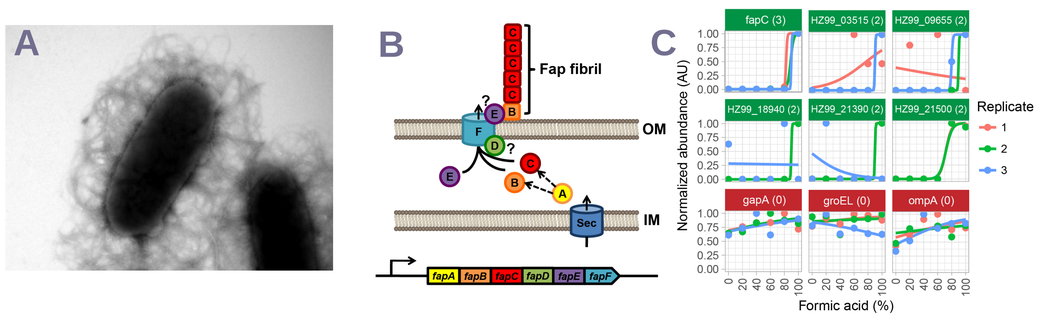
RESULTS

Escherichia coli SM2258 producing curli functional amyloids



A) TEM image of *E. coli* SM2258. **B)** Curly biosynthesis. **C)** Proteins with amyloid-specific abundance signatures in at least two biological replicates. Negative controls including the household proteins, Glyceraldehyde-3-phosphate dehydrogenase A (GapA) and 60 kDa chaperonin (GroEL); and β-barrel outer membrane proteins, like outer membrane protein A (OmpA) and antigen 43 (Ag43).

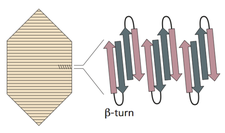
Pseudomonas sp. UK4 producing Fap functional amyloids



A) TEM image of *Pseudomonas sp. UK4* . **B)** Fap biosynthesis. **C)** Proteins with amyloid-specific abundance signatures including the major functional amyloid subunit (FapC) and negative controls.

Activated sludge spiked with 1% *E. coli* producing curli

Cells from E. coli producing curli were mixed with activated sludge from a Danish WWTP. As expected, the profiles of the CsgA and CsgB monomers was found. Interestingly, another structurally confirmed functional amyloid was identified among the sludge proteins, namely the gas vesicle protein A, **GvpA**



GvpA

FINDINGS

- We have developed a technique that allows the direct identification of functional amyloids in cell lysates.
- The technique is general applicable and do not require detailed knowledge about the bacterial composition.
- The technique can be applied to complex samples e.g. clinical biofilms. It can therefore be used to identify amyloids produced by stimuli that can't be mimicked in the laboratory.
- We believe that the method will result in the identification of numerous new functional amyloids in the coming years.

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